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Acute, but not chronic, metabolic acidosis disturbs 25-hydroxyvitamin D_3 metabolism

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Acute, but not chronic, metabolic acidosis disturbs 25-hydroxyvitamin D_3 metabolism. Previous studies in vitamin D deficient animals showing that acidosis may impair the production of $1,25(OH)_2D_3$ are in conflict with studies in humans which have not shown convincing disturbances of $25OHD_3$ metabolism during acidosis. We have investigated the effect on renal $25OHD_3$ 1- and 24-hydroxylase of acid loading for periods of 24 hr to 21 days. Acid loading resulted in immediate and sustained decrements in arterial pH and bicarbonate and increments in blood-ionized calcium. Acute (24-hr) acid loading decreased 1- and increased 24-hydroxylase activity. After 6 days of acid loading, no effect on 1-hydroxylase activity was observed and that on 24-hydroxylase activity was reduced. By 21 days the effect of acidosis on 24-hydroxylase activity was no longer observed. The results show that acidosis disturbs $25OHD_3$ metabolism in the physiological vitamin D and calcium replete state as well as in the vitamin D deficient state, but only acutely. Our data are consistent with studies in humans and suggest that, while short-term disturbances of $25OHD_3$ metabolism occur early in acidosis, they are transient and may not be causally related to the development of metabolic bone disease during chronic acidosis.

L'acidose métabolique aiguë et non chronique perturbe le métabolisme de la 25-hydroxyvitamine D_3. Des études antérieures chez des animaux déficients en vitamine D indiquant que l'acidose pourrait altérer la production de $1,25(OH)_2D_3$ sont en conflit avec des études chez les humains, qui n'ont pas montré d'anomalie convaincante du métabolisme de la $25OHD_3$ pendant l'acidose. Nous avons étudié l'effet sur la $25OHD_3$ 1- et 24-hydroxylase rénale d'une charge acide pendant des périodes de 24 heures à 21 jours. La charge acide a entraîné des diminutions immédiates et prolongées du pH artériel et des bicarbonates, et des augmentations du calcium ionisé sanguin. Une charge aiguë (24 heures) a diminué l'activité de la 1- et augmenté celle de la 24-hydroxylase. Après 6 jours de charge acide, aucun effet sur l'activité 1-hydroxylase n'a été observée, et celui sur l'activité 24-hydroxylase était réduit. Au bout de 21 jours, l'effet de l'acidose sur l'activité 24-hydroxylase n'était plus visible. Ces résultats montrent que l'acidose altère le métabolisme de la $25OHD_3$ lors d'un état physiologique en vitamine D et en réplétion calcique, comme dans un état de déficience vitaminique D, mais seulement de façon aiguë. Nos données sont compatibles avec les études chez les humains et suggèrent que bien que des altérations du métabolisme de la $25OHD_3$ à court terme puissent se produire tôt en acidose, celles-ci sont transitoires, et ne sont probablement pas reliées au développement d'une ostéopathie métabolique pendant l'acidose chronique.

The similarity between the skeletal abnormalities observed in acidotic states and those of vitamin D deficiency or in subjects with disordered vitamin D metabolism has led to the suggestion that an acidosis-induced disturbance of vitamin D metabolism may contribute to the genesis of this type of bone disease [1, 2]. Studies in vitamin D-depleted chicks and rats have shown that acidosis induced by feeding ammonium chloride (NH_4Cl) is associated with decreased conversion of 25-hydroxyvitamin D_3 ($25OHD_3$) to 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], both in vivo [3–5] and in vitro [4, 6, 7]. If these apparent disturbances of $25OHD_3$ metabolism are a direct consequence of the acid loading, the possibility of diminished production of $1,25(OH)_2D_3$ leading to widespread alterations of mineral and skeletal metabolism exists. Alternatively, if the change in $25OHD_3$ metabolism is merely a compensation for other effects of acidosis on mineral metabolism, such as increased ionized calcium concentration, it is much less likely that acidosis-induced alterations of $25OHD_3$ metabolism are causally related to metabolic bone disease seen in chronically acidotic humans and animals.

Although impairment of $1,25(OH)_2D_3$ production during acidosis has been shown in vitamin D replete animals, this effect could only be demonstrated under conditions of calcium deprivation [8], and it is not known whether acidosis may disturb $25OHD_3$ metabolism in the more physiological vitamin D and calcium replete states.

The acute animal studies described above have yielded results different from those of the effect of induced [9–11] or spontaneous [12] metabolic acidosis in humans. These studies have not shown measurably disturbed vitamin D metabolism during acidosis.

In an attempt to resolve some of these questions, we have performed experiments using both vitamin D deficient and replete rats and have endeavored to relate the disturbed $25OHD_3$ metabolism associated with acid loading to the chronicity of the acidosis and to concomitant changes in pH, calcium, and inorganic phosphorus.

Methods

Animals. Male Lewis rats (Charles River Laboratories, Wilmington, Massachusetts) were placed on a vitamin D deficient diet containing 0.2% calcium and 0.4% phosphorus (Teklad, Madison, Wisconsin) at the time of weaning. The animals were reared in the dark and studied after 7 weeks on the diet. Some

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experiments were performed using Lewis rats reared on a nutritionally complete diet containing vitamin D 113 μg/kg, 1.2% calcium, and 0.8% phosphorus (Diet 5001, Ralston Purina, St. Louis, Missouri) until they were 10 weeks old.

Acidosis was induced by giving drinking water containing either 0.3 M NH_4Cl (to vitamin D deficient rats) or 0.4 M NH_4Cl (to vitamin D replete rats) for periods of 1, 6, or 21 days prior to experimentation. To eliminate interassay variation and to minimize errors arising from the use of different batches of animals, each treatment group was compared with a simultaneously studied control group.

Enzyme assays. Direct assays of mitochondrial $25OHD_3$ 1- and $25OHD_3$ 24-hydroxylase activity were performed separately on the mitochondria from each animal by a method adapted from that of Vieth and Fraser [13]. This technique has been shown to result both in a linear time course of product formation up to 20 min for 1-hydroxylase and to 30 min for 24-hydroxylase and in a linear relationship between $1,25(OH)_2D_3$ or 24,25-dihydroxyvitamin D [$24,25(OH)_2D_3$] production and mitochondrial protein concentration up to at least 5 mg/ml [13]. The kidneys were removed, weighed, stripped of connective tissue and fat, diced and homogenized in 15 vol (v/w) of ice-cold buffer (250 mM sucrose, 10 mM KCl, 10 mM Hepes, and 2 mM EGTA) with the pH adjusted to 7.4 at 25°C. The homogenate was centrifuged at ×4,000*g* for 40 sec and the supernatant recentrifuged at ×9,000*g* for 20 min. The resulting pellet, consisting mainly of mitochondria, was resuspended in ice-cold incubation medium (125 mM KCl, 20 mM Hepes, 10 mM L-malic acid, 2 mM $MgSO_4$, and 0.25 mM EGTA, pH 7.0 at 25°C). Prior to incubation, mitochondrial protein concentration was adjusted to approximately 4 mg/ml pending definitive assay [14]. Incubations were carried out in a shaking incubator at 25°C using 1 ml of mitochondrial suspension gassed with 100% O_2. After a 3-min preincubation, 500 pmoles of $25OHD_3$ containing 20,000 dpm of 26,27-^{3}H $25OHD_3$ were added in 20 μl of acetone. The reaction was terminated after 20 min by the addition of 3.75 ml of methanol:chloroform (2:1), containing 0.017% butylated hydroxytoluene. Appropriate blanks using boiled mitochondria were included in all experiments.

Extraction, separation, and identification of metabolites. In all experiments lipid extraction was by a modified [13] method of Bligh and Dyer [15]. Separation of radioactive vitamin D metabolites was accomplished by high pressure liquid chromatography (HPLC), using a Rad-Pak Sil 0.5 × 10 cm analytical column with 10 μm packing (Waters Associates, Milford, Massachusetts), eluted with methylene chloride:methanol (98:2), at a flow rate of 3 ml/min. Solvent delivery was by a model 6000A pump linked to a U6K injector and to a model 440 UV detector (all equipment from Waters Associates). Fractions of 0.75 ml were collected and evaporated; radioactivity was determined by liquid scintillation spectroscopy. In all HPLC separations a Corasil II Guard Column was used to protect the analytical column. Radioactive peaks were identified provisionally by co-chromatography with authentic unlabelled $25OHD_3$, $24,25(OH)_2D_3$, 25,26-dihydroxyvitamin D_3 [$25,26(OH)_2D_3$], $1,25(OH)_2D_3$, and 1,24,25-trihydroxyvitamin D_3 [$1,24,25(OH)_3D_3$]. The putative $1,25(OH)_2D_3$ peak was rechromatographed on a different straight phase system using a 3.9 mm × 36 cm μ-Porasil column (Waters Associates) eluted with hexane:isopropanol (90:10). The putative $24,25(OH)_2D_3$ peak was subjected to treatment with sodium metaperiodate [16] and also to rechromatography on a μ-Porasil column eluted with methylene chloride:isopropanol (99:1).

Calculations. Preliminary experiments demonstrated that the recovery of tritiated $25OHD_3$, $24,25(OH)_2D_3$, and $1,25(OH)_2D_3$ following the extraction and initial straight phase HPLC described above was essentially the same for each of the three metabolites, averaging 87%. This allowed the calculation of enzyme activity from the amount of radioactivity appearing as product expressed as a fraction of total radioactivity recovered. The amount of product formed was corrected for mitochondrial protein concentration. Enzyme activity was expressed as femtomoles of product produced per milligram of mitochondrial protein per minute. Group data are expressed as the mean ± SEM. Student's *t* test for unpaired data was used to determine the significance of differences between groups; a value of $P < 0.05$ was regarded as significant.

Chemical determinations. All radiolabelled compounds were purchased from Amersham Searle, Arlington Heights, Illinois. Nonradioactive $25OHD_3$ was a gift from Upjohn (Kalamazoo, Michigan). The $24,25(OH)_2D_3$, $25,26(OH)_2D_3$, $1,25(OH)_2D_3$, and $1,24,25(OH)_3D_3$ were gifts from Dr. M. R. Uskokovic of Hoffmann-LaRoche (Nutley, New Jersey). All other chemicals and solvents were commercially supplied. Total calcium [17] and inorganic phosphorus [18] were measured in plasma as previously described. Blood-ionized calcium was measured with an ionized calcium analyzer (Model SS-20, Orion Biomedical, Cambridge, Massachusetts). Arterial pH and P_{CO_2} were measured using a blood gas analyzer (Corning 175, Corning Medical, Medfield, Massachusetts) and bicarbonate concentration was calculated from the Henderson Hasselbach equation.

Results

Characterization of vitamin D metabolites. Typical chromatograms of lipid extracted after incubation with mitochondria from vitamin D deficient and vitamin D replete rats are shown in Figure 1. When the $1,25(OH)_2D_3$ peak from the initial straight phase HPLC using the Rad-Pak Sil column was rechromatographed on a μ-Porasil column eluted with hexane:isopropanol (90:10), 82% of the applied radioactivity comigrated with authentic unlabelled $1,25(OH)_2D_3$ (Fig. 2). Treatment of the $24,25(OH)_2D_3$ peak from the initial straight phase HPLC with sodium metaperiodate resulted in loss of 85% of the lipid extractable radioactivity. When the $24,25(OH)_2D_3$ peak was rechromatographed on a μ-Porasil column eluted with methylene chloride:isopropanol (99:1), 79% of the applied radioactivity was recovered comigrating with authentic unlabelled $24,25(OH)_2D_3$ (Fig. 2).

Mitochondria from vitamin D deficient rats produced significant amounts of $1,25(OH)_2D_3$ but no detectable $24,25(OH)_2D_3$, $25,26(OH)_2D_3$, or $1,24,25(OH)_3D_3$. Conversely, mitochondria from vitamin D replete rats produced $24,25(OH)_2D_3$ and only very small amounts of material which comigrated with authentic $25,26(OH)_2D_3$ and $1,25(OH)_2D_3$ on initial straight-phase HPLC. Rechromatography of these materials on a μ-Porasil column eluted with hexane:isopropanol (90:10), resulted in a loss of 60 to 75% of radioactivity comigrating with authentic standards. Thus, the production of $25,26(OH)_2D_3$ and $1,25(OH)_2D_3$ by mitochondria from vitamin D replete rats was minimal and could not be quantitated reliably.

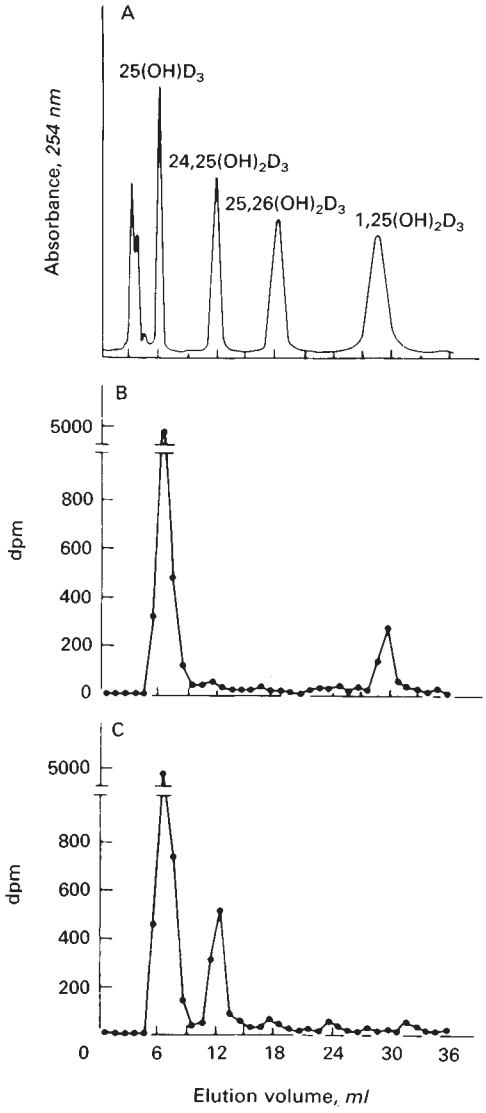

Fig. 1. **A** *HPLC profile of unlabelled standards,* **B** *labelled metabolites following incubations with mitochondria from vitamin D deficient rats,* and **C** *following incubations with mitochondria from vitamin D replete rats.* A Rad Pak Sil radial compression column was eluted with methylene chloride:methanol (98:2) at 3 ml/min (see **Results**).

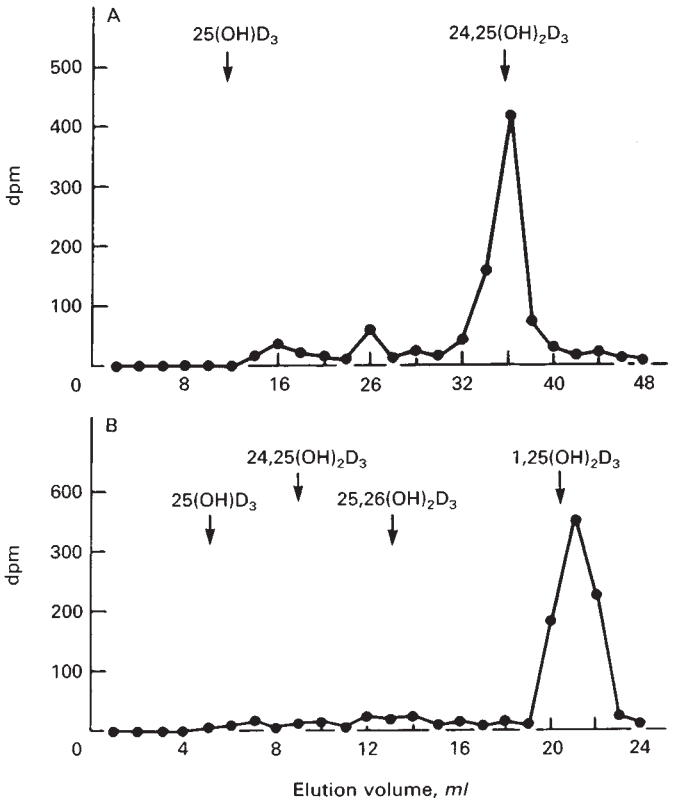

Fig. 2. **A** *Rechromatography of the putative 24,25(OH)$_2$D$_3$ peak from the initial HPLC shown in Figure 1***C**. The μ-Porasil column was eluted with methylene chloride:isopropanol (99:1) at 2 ml/min. **B** Rechromatography of the putative 1,25(OH)$_2$D$_3$ from the initial HPLC shown in Figure 1**B**. The μ-Porasil column was eluted with hexane:isopropanol (90:10) at 2 ml/min. *Vertical arrows* indicate the positions of unlabelled standards.

Effect of acid loading. In all experiments, NH_4Cl loading induced a marked and sustained acidosis as manifest by decreases in blood pH and bicarbonate concentration (Table 1).

In vitamin D-depleted rats, acute (24 hr) acid loading was associated with a marked increment in plasma ionized calcium (3.5 ± 0.2 vs. 2.5 ± 0.2 mg/dl, $P < 0.001$). Plasma total calcium increased in parallel with ionized calcium (6.6 ± 0.3 vs. 5.4 ± 0.4 mg/dl, $P < 0.002$). Plasma phosphate did not change significantly (Table 1). In the control animals, 25OHD$_3$ 1-hydroxylase activity, measured separately in each animal, was 105 ± 17 fmoles min^{-1} mg^{-1}, a value that agrees with previous work [19]. Acute (24-hr) acid loading resulted in a substantial decrement in 1-hydroxylase from 105 ± 17 fmoles min^{-1} mg^{-1} in the control animals to 66 ± 8 fmoles min^{-1} mg^{-1} in the acidotic animals (Fig. 3).

In contrast, after 6 days of acid loading, the suppression of 1-hydroxylase in the acidotic animals was no longer observed (95 ± 12 fmoles min^{-1} mg^{-1} in the control animals versus 84 ± 12 fmoles min^{-1} mg^{-1} in acidotic animals) despite higher plasma ionized calcium concentrations in the acidotic animals. Plasma phosphate was not altered significantly by NH_4Cl feeding for 6 days.

In vitamin D and calcium replete rats, a similar tendency of acid loading to elevate plasma ionized calcium was seen (Table 1). As was the case with the vitamin D deficient animals, the experiments using vitamin D replete rats showed a time-dependent influence of acid loading on 25OHD$_3$ metabolism. In these animals, 24,25(OH)$_2$D$_3$ was the only metabolite produced in measurable quantities (Fig. 1). After 24 hr of acid loading, blood-ionized calcium was elevated significantly (5.1 ± 0.1 vs. 4.5 ± 0.1 mg/dl, $P < 0.001$), although total plasma calcium did not differ significantly between acidotic and control groups. Plasma phosphate decreased slightly following acute acid loading. These changes were accompanied by a marked increase in 24-hydroxylase activity from 143 ± 68 to 530 ± 59 fmoles min^{-1} mg^{-1}, $P < 0.001$ in the acidotic animals (Fig. 3). After 6 days, the augmentation of 24-hydroxylase by acidosis was reduced, though still significant (207 ± 30 vs. 119 ± 10 fmoles min^{-1} mg^{-1}, $P < 0.025$), despite an increment in plasma ionized

Table 1. Effect of ammonium chloride loading on pH, bicarbonate (HCO_3^-), ionized calcium (Ca^{++}), total calcium (Ca^t), and inorganic phosphorus (Pi) in rats[a]

	N	pH	HCO_3^-	Ca^{++}	Ca^t	Pi
Vitamin D deficient 24-hr acidosis						
Control	8	7.35 ± 0.03	25.1 ± 0.5	2.5 ± 0.2	5.4 ± 0.4	6.5 ± 0.5
		$P < 0.001$	$P < 0.001$	$P < 0.01$	$P < 0.02$	NS
Acidotic	10	7.15 ± 0.04	13.2 ± 1.3	3.5 ± 0.2	6.6 ± 0.3	6.3 ± 0.2
Vitamin D deficient 6-day acidosis						
Control	6	7.27 ± 0.02	23.8 ± 0.8	2.0 ± 0.1	4.9 ± 0.2	7.0 ± 0.6
		$P < 0.02$	$P < 0.01$	$P < 0.05$	NS	NS
Acidotic	8	7.16 ± 0.03	15.0 ± 1.2	2.7 ± 0.3	5.6 ± 0.3	5.6 ± 0.3
Vitamin D replete 1-day acidosis						
Control	8	7.39 ± 0.01	28.0 ± 0.7	4.5 ± 0.1	10.6 ± 0.2	8.9 ± 0.3
		$P < 0.001$	$P < 0.001$	$P < 0.001$	NS	$P < 0.02$
Acidotic	12	7.23 ± 0.02	15.0 ± 0.6	5.1 ± 0.1	10.9 ± 0.1	7.7 ± 0.3
Vitamin D replete 6-day acidosis						
Control	8	7.42 ± 0.01	28.0 ± 0.8	4.5 ± 0.1	10.3 ± 0.1	8.1 ± 0.4
		$P < 0.001$	$P < 0.001$	$P < 0.001$	NS	$P < 0.01$
Acidotic	8	7.21 ± 0.02	15.8 ± 0.4	5.2 ± 0.1	10.5 ± 0.1	6.7 ± 0.2
Vitamin D replete 21-day acidosis						
Control	8	7.35 ± 0.02	27.3 ± 0.6	4.5 ± 0.1	10.9 ± 0.2	8.0 ± 0.2
		$P < 0.01$	$P < 0.001$	$P < 0.001$	$P < 0.05$	$P < 0.02$
Acidotic	10	7.26 ± 0.02	20.0 ± 0.6	5.1 ± 0.1	10.3 ± 0.2	7.1 ± 0.2

[a] Vitamin D deficient and replete rats were given ammonium chloride for periods of 24 hr, 6 days, or 21 days. Data shown are mean ± SEM of measurements made on aortic blood samples.

calcium and severity of acidosis similar to that seen at 24 hr. Total calcium was comparable in control and acidotic animals, but plasma phosphate was decreased slightly by 6 days of acid loading. By 21 days, although the tendency of acid loading to elevate ionized calcium persisted and was comparable to that at 24 hr and 6 days, the augmentation of 24-hydroxylase activity by acidosis was no longer observed (137 ± 19 vs. 152 ± 29 fmoles min^{-1} mg^{-1} in control and acidotic animals, respectively). Plasma total calcium and phosphate in rats that had been acid-loaded for 21 days were both slightly lower than in control rats.

Discussion

These results show that the responses of the renal $25OHD_3$ 1- and 24-hydroxylases to acute experimentally induced acidosis are qualitatively different from those seen after more prolonged acidosis of 6 or more days duration. The data show that acidosis-induced changes in $25OHD_3$ metabolism are not confined to vitamin D and calcium-depleted animals but are demonstrable in the more physiological vitamin D and calcium replete state as well.

The control of $25OHD_3$ metabolism to $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ is mediated by a variety of hormonal [20–25] and ionic [26–29] factors, of which calcium, phosphate, and PTH are probably the most important. Metabolic acidosis appears to suppress 1-hydroxylase by inhibiting PTH sensitive adenylate cyclase without affecting post c-AMP events [6]. However, little is known of the potential of the renal $25OHD_3$ hydroxylases to lose their responsiveness to a perturbation that is maintained for an extended period, although other aspects of renal function do exhibit adaptive responses to certain conditions, such as acid loading [30–32].

Our demonstration of a clear-cut suppression of 1-hydroxylation of $25OHD_3$ during short-term (24 hr) acidosis, with an accompanying increase in ionized calcium from 2.5 to 3.5 mg/dl, without changes in plasma phosphate, suggest calcium as a likely mediator of the suppression of 1-hydroxylase by acidosis. Previous studies in vitamin D deficient chicks have shown that modest increments in plasma calcium, induced by increasing dietary calcium, caused marked suppression of 1-hydroxylase activity, although calcium rose only slightly and remained in the hypocalcemic range [33, 34].

When we studied rats that had been acid-loaded for 6 days, the initial suppression of 1-hydroxylase, seen at 24 hr, was no longer apparent. This change occurred although blood-ionized calcium remained elevated in the acidotic animals and the severity of acidosis was comparable to that at 24 hr.

The experiments done on vitamin D replete animals showed that the 24-hydroxylase exhibited a time course of adaptation similar to that of 1-hydroxylase. In these rats, acute (24 hr) acidosis induced very large increases in 24-hydroxylase activity, with an associated increase in blood-ionized calcium from 4.5 to 5.1 mg/dl. Parathyroidectomy has been shown to increase 24-hydroxylase activity in vitamin D replete rats [19], and it is possible that the increment in ionized calcium in the acidotic animals, by decreasing PTH production, increases 24-hydroxy-

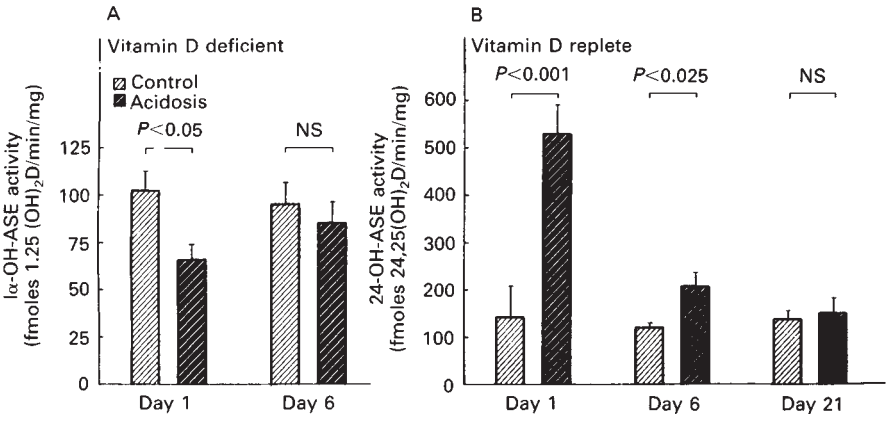

Fig. 3. A *In vitro kidney 25OHD$_3$ 1-hydroxylase activity in vitamin D deficient rats after ammonium chloride loading for 24 hr or 6 days.* Incubations of mitochondria from each animal were performed in duplicate. *Vertical bars* represent the mean ± SEM of values obtained from 6 to 12 rats per group. **B** *In vitro kidney 24-hydroxylase activity in vitamin D replete rats after ammonium chloride loading for 24 hr, 6 days, or 21 days.* Data are the mean ± SEM of duplicate incubations performed separately on each of 8 to 12 rats per group.

lase activity by this mechanism. Furthermore, the anticalciuric action of PTH on the kidney is impaired during metabolic acidosis [35] and, although an effect of acidosis on target organ responsiveness to PTH has been questioned recently [36], it is possible that the normal action of PTH to stimulate 1-hydroxylase and suppress 24-hydroxylase is impaired by acidosis. After acid loading for 6 days, the influence of acidosis on ionized calcium was the same, but that on 24-hydroxylase activity was much less. After 21 days, the increment in ionized calcium in the acidotic animals was still the same as at earlier time points, but there was now no effect of acidosis on 24-hydroxylase activity.

The increments in blood-ionized calcium that we have documented during acidosis were marked and may have resulted from decreased binding of calcium to plasma protein, or from the entry of a large amount of skeletal calcium into the extracellular fluid as the acid load was partially buffered by alkaline bone salts [37]. Plasma phosphate was slightly lower in the acidotic animals and therefore hyperphosphatemia cannot be implicated as a mediator of the effects of acidosis. The finding that acidosis leads to reciprocal changes of 1- and 24-hydroxylase activity indicates that a direct toxic effect of NH_4Cl or acidosis on the 25OHD$_3$ hydroxylases is an unlikely explanation for our observations.

The complementary adaptive changes in two closely related enzyme systems, occuring within 6 days of the onset of acidosis, does not agree with certain previous studies. Thus, Kawashima, Kraut, and Kurokawa [6], using dissected rat nephron segments and Reddy et al [7], using isolated perfused rat kidneys, have shown decreased 1-hydroxylase in vitro following 7 and 9 days, respectively, of NH_4Cl loading. Furthermore, Kawashima's studies showed unimpaired 1-hydroxylation of 25OHD$_3$ after acute (16 hr) acidosis. The reason for these inconsistencies is unclear, although the differences between the assay techniques used may be important.

It is known that the kidney may adapt to chronic acidosis in several ways, including macroscopic hypertrophy [30], stimulation of renal gluconeogenesis [31], and increased renal ammonia excretion [32]. These changes do not occur instantaneously but may take several days to manifest fully [32]. However, although these adaptive phenomena show a time course which approximates the adaptation of the 25OHD$_3$ 1-hydroxylases during acid loading, we can only speculate on their relationship to the mechanism by which the hydroxylases adapt. That the adaptation occurs while blood-ionized calcium, pH, bicarbonate and inorganic phosphate concentrations are constant suggests that some other parameter, or parameters, of the acidotic state must operate early, but not late, in acidosis to mediate the influence of acidosis on the metabolism of 25OHD$_3$. Alternatively, an initial influence of acidosis (either direct or indirect) on the renal 25OHD$_3$ hydroxylases may subsequently be opposed by delayed compensatory mechanisms which become more dominant as the duration of acid loading increases.

Our results, with recent data from Bushinsky et al [8] showing that suppression of plasma $1,25(OH)_2D_3$ in the acid-loaded rat is only apparent when the 1-hydroxylase has been stimulated previously by dietary calcium restriction, may explain why disturbances of vitamin D metabolism have not been seen in chronically acidotic, vitamin D, and calcium replete humans. The present studies are compatible with data obtained from volunteers with induced acidosis [9–11] and from patients with severe spontaneous acidosis [12]. The results support the view that, while short-term disturbances of 25OHD$_3$ metabolism may occur during the development of acidosis, such disturbances are transient and probably not causally related to the development of metabolic bone disease during chronic acidosis.

Acknowledgments

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